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Publication date:
2013

Document Version
Publisher's PDF, also known as Version of record

A study of the Gamma hypothesis: Predicting combined effects of pH and salt on growth of Listeria monocytogenes

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OBJECTIVE(S)

Controlling factors to limit microbial growth are frequently applied in foods to achieve an overall level of protection against foodborne pathogens and spoilage flora. To assess the combined effect of the controlling factors on microbial growth, some predictive models assume independent additive effect of the controlling factors [1] (Gamma hypothesis: absence of synergistic effects), whereas other models apply the socalled LeMarc interaction term [2] to quantify possible synergistic effects between the controlling factors near the growth boundary. The purpose of this study was to investigate if synergistic effects on growth of *L. monocytogenes* exist between the controlling factors pH and salt, and to investigate if the LeMarc interaction term is a suitable method to quantify this effect in a model, and if the term possible could be improved.

METHODS(S)

The growth mediums used for the growth experiments were BHI broth prepared with varying NaCl concentrations (0-9 w/w%), and varying pH (4-8) according to a complete factorial experimental design. Growth of *L. monocytogenes* strain ATCC19115 was followed by OD600 measurements in an automatic reader Bioscreen C system (Lab systems Corp. Helsinki, Finland). The maximum specific growth rates for each combination of salt and pH were derived from the OD growth curves. From the experimental data independent secondary models for influence of pH and salt on growth of *L. monocytogenes* were derived and the performance of each independent secondary model was compared. The best performing independent secondary models were included in combined non-synergy and synergy models and their performance were compared when tested on the experimental data. One of the synergy models includes a novel interaction term. The best performing combined model was finally validated on a separate data set.

RESULTS

The results show that the combined model including the LeMarc interaction term performed better than the non-synergy model, and the model with the novel interaction term performed even better for *L. monocytogenes*, when a threshold value in the novel interaction term was adjusted so that the synergistic effect commence at a lower level of combined environmental stress than prescribed in the LeMarc interaction term.

CONCLUSIONS AND IMPACT OF THE STUDY

Our results confirm that there are synergistic effects of pH and NaCl near the growth boundary for *L. monocytogenes* and that the LeMarc interaction term is a suitable way to describe the combined inhibiting effect, even though the model using the novel interaction term performed better for our data. Our interpretation is that the arbitrary threshold value of combined environmental stress of 0.5 in the LeMarc interaction term controlling when synergy appear between controlling happens, is not a general number, but happens to be suitable for *L. monocytogenes*, even though our results indicate that a value of 0.39 is better. Other studies have tried to include the LeMarc interaction term in models for other bacteria species, but did obtain better results, when adding the LeMarc interaction term to their model of *Bacillus cereus* [3]. A future hypothesis to investigate could be that all bacterial species are

actually affected by synergistic effects of controlling factors, but they are maybe not equally sensitive to level of combined environmental stress at which synergy commence as conditioned in the LeMarc interaction term. Future research could quantify this possible difference in sensitivity between different bacteria using the novel modified interaction term.

REFERENCES

1. *Zwietering et al 1992, Journal of Food Protectin, 55, 973-979*
2. *LeMarc et al. 2002, Int.J.Food Microbiology, 73, 219-237*
3. *Biesta-Peters et al. 2010, Applied and Environmental Microbiology, 76, 5791-5801*